acterized as having a small trapping volume, a low trapping efficiency (10%) and a confined aqueous space (15 to 35 A).

Batzri and Korr, Biochim. Biophys. Acta, 298:1015 (1973) using ethanol, and Deamer and Bangham Biochim. Biophys. Acta, 443:629–634 (1976) using ether, describe lipid vesicles prepared by injection of lipids in an organic phase into an aqueous solution. These methods produce unilamellar or paucilamellar vesicles.

U.S. Pat. No. 4,235,871 to Papahadjopoulos et al describes a method of encapsulating biologically active materials in synthetic, oligolamellar, lipid vesicles which comprises: providing a mixture of a vesicle wall forming compound in organic solvent and an aqueous mixture of the biologically active material to be encapsulated, the ratio of organic phase to aqueous phase being that which will produce an emulsion of the water-in-oil type; forming a homogeneous water-in-oil type of emulsion of said mixture; evaporating organic solvent from the emulsion, until a mixture is obtained having a gel-like character; and converting the gel-like mixture to a suspension of synthetic, oligolamellar vesicles encapsulating the biologically active material by one of the steps of (a) agitating said gel-like mixture and (b) dispersing said gel-like mixture in an aqueous media.

U.S. Pat. No. 4,429,008 to Martin et al describes a composition useful for conjugation with ligands bearing thiol groups, wherein each liposome has a lipid bilayer defining an outer surface for the liposome, and a plurality of thiol reactive groups integrally connected to the lipid bilayer and extending outward with respect to the outer surface.

Methods of associating antibodies with liposomes have been described and may be generally divided into two groups; nonspecific association and covalent attachment. Nonspecific association appears to rely upon the affinity of the Fc portion of the antibody for the hydrophobic region of the lipid bilayer.

Heath et al Science vol 210:539–541 (1980), reported efficiently covalently binding liposomes to biologically active proteins by periodate oxidation of glycosphingolipids.

SUMMARY OF THE INVENTION

Briefly, the invention comprises a process for assaying an analyte, said analyte being a member of a specific binding pair consisting of ligand and antiligand, wherein the process comprises the steps of: obtaining a first fluid suspected of containing the analyte to be determined; combining the first fluid with a solid support which has been sensitized with receptors that will bind the analyte to be determined; contacting the support with a second fluid comprising ATP encapsulated within the walls of liposomes, said liposomes having bonded thereto a compound which is either a ligand, ligand analog, or antiligand; and testing for the presence of ATP.

DETAILED DESCRIPTION OF THE INVENTION

Before considering the subject invention in detail, a number of terms used in the specification will be defined:

Antigen: any substance capable of provoking an immune response, particularly with the production of specific antibodies, in vertebrates. They include proteins, glycoproteins, glycolipids, polysaccharides, lipopolysaccharides.

Hapten: an incomplete antigen, incapable of itself in provoking an immune response, but when suitably attached to another molecule, generally a protein, becomes capable of producing antibodies which will specifically recognize the hapten molecule.

Epitope: a specific chemical and spacial configuration which is specifically recognized by an antibody. Antigens usually have a plurality of epitopic sites.

Analyte: the compound to be measured, which can be a ligand that is mono or polyepitopic, antigenic or haptenic, a single or plurality of compounds which share at least one common epitopic site or a receptor. The analyte can also be a DNA probe

Ligand: any compound for which a receptor naturally exists or can be prepared.

Ligand analog: a modified ligand which can compete with the analogous ligand for a receptor, the modification providing means to join the modified ligand to another molecule.

Receptor: any compound capable of recognizing a particular spatial and polar organization of a molecule, i.e., epitopic site. They include antibodies, enzymes, antibody fragments such as Fab & Fab'2 fragments, DNA fragments, lectins, complement components, conglutinin, rheumatoid factors, hormones, avidin, staphylococcal protein 'A, etc.

DNA probe: small pieces of DNA that recognizes specific genes by hybridizing to complementary DNA.

Label (Marker): a compound which is either directly or indirectly involved with the production of a detectable signal.

Sac: a bag of any material enclosing a volume, having a wall composed of one or more components and having at least one internal compartment with the wall of the compartment forming a permeability barrier to the outside.

Vesicles: a term used to cover both single and multicompartmented sacs, but used herein solely to cover single compartment sacs.

Ghosts: sacs obtained from cells by removing cellular contents by opening the cellular membrane, either by physical or chemical means, so as to substantially empty the cell of its contents and then sealing the membrane, so as to enclose the material present in the sealing solution.

Liposomes: single or multicompartmented bodies obtained when lipids, particularly lipid mixtures, are dispersed in aqueous suspension. The walls or membranes are composed of a continuous lipid bilayer.

The high solubility and stability of ATP and the availability of inexpensive and stable luciferin-luciferase reagents provide an extremely sensitive method of identifying an immunological reaction of antigen or antibody coated liposome particles or a nucleic acid hybridization of a ligand-coupled DNA probe detected by antiligand-coated liposomes.

The presence of endogenous ATP in serum or urine samples and various inhibitors of the luciferin-luciferase reaction limits the reaction to a heterogeneous system, where immunologically bound liposomes must be separated from unbound liposomes. Fortunately there are a number of systems available to satisfy this requirement.

One such system is to use filtration, where the filtration membrane pore size is large enough to allow unbound liposomes to flow freely upon vacuum or pressure, but retains bound liposomes to larger particles which are also immunologically tagged.